

CLAIMS

What is claimed:

1 1. A method for preparing an tracer composition
2 comprising:

3 obtaining a ^{13}C labeled Krebs cycle metabolite
4 precursor that will produce an analyte;

5 obtaining a deuterium source;

6 wherein gluconeogenensis is measured from a subject
7 that was provided the precursor and the deuterium source,
8 and produced the analyte, by comparison of the relative
9 nuclear magnetic resonance profiles of the labeled
10 components in the analyte.

1 2. The method of claim 1, wherein the analyte is ^{13}C -
2 glucose.

1 3. The method of claim 1, wherein the presursor is
2 glucose, lactose, lactate or alanine.

1 4. The method of claim 1, wherein the deuterium
2 source is deuterated water.

1 5. The method of claim 1, wherein the analyte is
2 glucose deuterated in the 2, 5 and 6 positions, and any

3 transformation that maintains the 2,5 and 6 positions in
4 relation to one another.

1 6. The method of claim 1, wherein the analyte is (1-
2 6 $^{13}\text{C}_2$)-glucose.

1 7. The method of claim 1, wherein the water is D₂O.

1 8. The method of claim 1, wherein the flux is
2 measured from blood, urine or tissue extracts.

1 9. The method of claim 1, wherein the analyte is ^{13}C -
2 labeled glucose with the label at the 2 or 5 positions,
3 or at both positions.

1 10. The method of claim 9, wherein the metabolite is
2 a transformation of the labeled glucose containing the
3 labeled 2 position, or the labeled 5 position, or both.

1 11. The method of claim 1, further comprising the
2 step of adding $^{13}\text{C}_3$ -propionate.

1 12. The method of claim 1, wherein the Krebs cycle
2 precursor is selected from the group consisting of
3 pyruvic acid, acetic acid, acetoacetic acid, beta-

4 hydroxybutyric acid, a Krebs cycle pathway metabolite,
5 and mixtures thereof.

1 13. The method of claim 1, wherein the analyte is
2 selected from the group consisting of pyruvic acid,
3 acetic acid citric acid, isocitric acid, cis-aconitic
4 acid, 2-ketoglutaric acid, succinic acid, fumaric acid,
5 malic acid, oxaloacetic acid, and mixtures thereof.

1 14. A method for preparing an tracer composition
2 comprising:

3 obtaining a deuterium source;
4 wherein gluconeogenesis is measured from a subject
5 that was provided the deuterium source, and produced an
6 analyte, by comparison of the relative nuclear magnetic
7 resonance profiles of the deuterium components in the
8 analyte.

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1 15. The method of claim 14, wherein the deuterium
2 source is deuterated water.

1 16. The method of claim 14, wherein the analyte is
2 glucose deuterated in the 2, 5 and 6 positions, and any
3 transformation that maintains the 2,5 and 6 positions in
4 relation to one another.

1 17. The method of claim 14, wherein the analyte is
2 (1-6 $^{13}\text{C}_2$) -glucose.

1 18. The method of claim 14, wherein the flux is
2 measured from blood, urine or tissue extracts.

1 19. The method of claim 14, wherein the analyte is
2 selected from the group consisting of pyruvic acid,
3 acetic acid citric acid, isocitric acid, cis-aconitic
4 acid, 2-ketoglutaric acid, succinic acid, fumaric acid,
5 malic acid, oxaloacetic acid, and mixtures thereof.

1 20. A method for preparing an isotopic metabolic
2 flux tracer composition comprising:

3 providing a ^{13}C labeled Krebs cycle metabolite
4 precursor to a subject to prouce an analyte;
5 obtaining a sample from the subject; and

6 measuring the nuclear magnetic resonance of the
7 labeled tracers to determine the rate of gluconeogenesis.

1 21. The method of claim 20, wherein the analyte is
2 ^{13}C -glucose.

1 22. The method of claim 20, wherein the analyte is
2 glucose labeled with ^{13}C at positions 1 through 6, or
3 combinations of two or more at any position.

1 23. The method of claim 20, wherein the analyte is
2 $(1-6\ ^{13}\text{C}_2)$ -glucose.

3 24. The method of claim 20, wherein the sample is
4 from blood, urine or tissue extracts.

5 25. The method of claim 20, further comprising the
6 step of providing the subject with $^{13}\text{C}_3$ -propionate.

1 26. The method of claim 20, wherein the Krebs cycle
2 precursor is selected from the group consisting of
3 pyruvic acid, acetic acid, acetoacetic acid, beta-
4 hydroxybutyric acid, a Krebs cycle pathway metabolite,
5 and mixtures thereof.

1 27. The method of claim 20, wherein the analyte is
2 selected from the group consisting of pyruvic acid,
3 acetic acid citric acid, isocitric acid, cis-aconitic
4 acid, 2-ketoglutaric acid, succinic acid, fumaric acid,
5 malic acid, oxaloacetic acid, and mixtures thereof.

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1 28. The method of claim 20, wherein the ^{13}C Krebs
2 cycle precursor is provided orally.

1 29. A method for measuring metabolic flux in a
2 sample using an isotopic metabolic flux tracer
3 composition comprising:

4 providing the sample with a ^{13}C Krebs cycle
5 precursor, D₂O and acetaminophen;
6 obtaining an analyte from the sample; and
7 measuring the relative amounts of acetaminophen
8 glucuronide and phenylacetylglutamine in the analyte using
9 nuclear magnetic resonance.

10 30. The method of claim 29, wherein the precursor is
2 ^{13}C -glucose.

1 31. The method of claim 29, wherein the precursor is
2 glucose labeled with ^{13}C at positions 1 through 6, or
3 combinations of two or more at any position.

1 32. The method of claim 29, wherein the precursor is
2 ($1\text{-}6\ ^{13}\text{C}_2$) -glucose.

1 33. The method of claim 29, wherein the sample is
2 from blood, urine or tissue extracts.

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4 34. The method of claim 29, further comprising the
2 step of providing the subject with $^{13}\text{C}_3$ -propionate.

1 35. The method of claim 29, wherein the Krebs cycle
2 precursor is selected from the group consisting of
3 pyruvic acid, acetic acid, acetoacetic acid, beta-
4 hydroxybutyric acid, a Krebs cycle pathway metabolite,
5 and mixtures thereof.

6 36. The method of claim 29, wherein the Krebs cycle
2 precursor is selected from the group consisting of
3 pyruvic acid, acetic acid citric acid, isocitric acid,
4 cis-aconitic acid, 2-ketoglutaric acid, succinic acid,
5 fumaric acid, malic acid, oxaloacetic acid, and mixtures
6 thereof.

1 37. The method of claim 29, wherein the ^{13}C Krebs
2 cycle precursor and D₂O are provided orally.

1 38. The method of claim 29, wherein the ^{13}C Krebs
2 cycle precursor and D₂O are provided to a mammal.

1 39. The method of claim 29, wherein the ^{13}C Krebs
2 cycle precursor and D₂O are provided to a human.

1 40. A reagent kit for use in effecting a
2 simultaneous assay for gluconeogenesis in a sample, said
3 reagent kit comprising:
4 a ^{13}C labeled Krebs cycle precursor; and
5 a labeled water tracer.

1 41. The reagents of claim 40, wherein the Krebs
2 cycle precursor is ^{13}C -glucose.

1 42. The reagents of claim 40, wherein the Krebs
2 cycle precursor is (1-6 $^{13}\text{C}_2$)-glucose.

1 43. The method of claim 40, wherein the Krebs cycle
2 precursor is glucose labeled with ^{13}C at positions 1
3 through 6, or combinations of two or more at any
4 position.

1 44. The reagents of claim 40, wherein the water
2 tracer is D₂O.

1 45. The reagents of claim 40, wherein the Krebs
2 cycle precursor is ¹³C₂-labeled glucose.

3 46. The reagents of claim 40, further comprising
2 ¹³C₃-propionate.

1 47. The reagents of claim 40, further comprising
2 acetaminophen.

1 48. The reagents of claim 40, further comprising an
2 acetaminophen glucuronide and/or an phenylacetylglutamine
3 standard.

1 49. The reagents of claim 40, wherein compositions
2 are prepared for oral administration.

1 50. The reagents of claim 40, wherein the Krebs
2 cycle precursor is selected from the group consisting of
3 pyruvic acid, acetic acid, acetoacetic acid, beta-
4 hydroxybutyric acid, a Krebs cycle pathway metabolite,
5 and mixtures thereof.

1 51. The reagents of claim 40, wherein the pH of the
2 components of the reagent kit is from about 3 to about 7.

1 52. The reagent kit of claim 40, further comprising
2 a buffered isotonic solution.

1 53. The reagent kit of claim 40, further comprising
2 a buffered isotonic solution wherein the buffer comprises
3 sodium borate and potassium cyanide.

1 54. A method for determining gluconeogenesis
2 comprising the steps of:

3 providing a patient with a ¹³C labeled Krebs cycle
4 precursor and D₂O;

5 obtaining a sample a blood, urine or tissue sample from
6 the patient;

7 measuring the ²H signal nuclear magnetic resonance
8 spectra;

9 measuring the ¹H NMR nuclear magnetic resonance spectra;

10 measuring the ¹³C-carbon nuclear magnetic resonance
11 spectra; and

12 calculating the rate of gluconeogenesis by taking the
13 known infusion rate of a ¹³C radiolabelled Krebs cycle

14 metabolite divided by the average fraction found in the
15 sample over a predetermined period.

1 55. The method of claim 54, therein the
2 predetermined time period is between about 2 to between
3 about 3 hours.

1 56. The method of claim 54, therein the
2 predetermined time period comprises measurements at 120,
3 150 and 180 minutes.

1 57. The method of claim 54, wherein the patient
2 fasts for 6-8 hours before taking the ¹³C labeled Krebs
3 cycle precursor and D₂O.

1 58. The method of claim 54, wherein the patient is
2 further provided with ¹³C propionate.

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